ABSTRACT

Commercially available radioimmunoassays (RIAs) are frequently used in toxicological studies to evaluate effects of endocrine disrupting chemicals (EDCs) on steroidogenesis in rats. Currently there are limited data comparing steroid concentrations in rats as measured by RIAs to those obtained using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). This study evaluates the concordance of serum and urine steroid concentrations as quantified by select RIA kits used in our laboratory and LC-MS/MS following exposure to an EDC, atrazine (ATR), at a dose known to produce elevations in a number of steroids. Adult ale rats were dosed with ATR (200 mg/kg/d) or methylcellulose (solvent control) by oral gavage gavage for 5 days. Animals were decapitated 2 hours after the final dose. Serum was collected and seperated into 2 aliquots for analysis. Serum was assayed by RIA for androstenedione (ANDRO), corticosterone (CORT), estradiol (E2), estrone (E1), progesterone (P4), and testosterone (T). Serum was extracted via solid phase extraction prior to LC-MS/MS analysis with positive electrospray ionization in multiple-reaction monitoring mode for A, CORT, P4, and T. E1 and E2 serum concentrations were quantified similarly by LC-MS/MS, following derivatization with dansyl chloride. To compare CORT values from urine, pregnant adult rats were dosed with either ATR (100 mg/kg/d) or methylcellulose by oral gavage for 5 days (i.e., gestational days 14-18). Urine samples were collected daily for 2 consecutive 6 hour intervals preceding and following dosing and assayed for CORT by RIA and LC-MS/MS as described above. Data analyses demonstrated a moderate to strong agreement between the two detection methods as assessed by Pearson product-moment correlation coefficient, Bland-Altman analysis, and the interclass correlation coefficent. No statistically significant differences were observed between RIA and LC-MS/MS means for any of the steroids assayed. These findings indicate

that steroids may be reliably measured in rat biological media using RIAs or LC-MS/MS in toxicological studies.

INTRODUCTION

An endocrine disrupting chemical (EDC) is defined as an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations (WHO, 2002). As presented in the 2012 review by De Coster and van Larebeke, disruptions of the endocrine system have been linked to numerous pathophysiologies including the development of hormone-dependent cancers, adverse effects on sexual development and reproductive function, and metabolic disorders (De Coster et al., 2012). Atrazine (ATR), a chlorotriazine herbicide used frequently within the United States to control grassy and broadleaf weeds for agricultural and landscape purposes, has been reported to cause adverse effects on the reproductive system in the laboratory rat (Eldridge et al., 1994; Wetzel et al., 1994; Cooper et al., 1996). Studies conducted by Cooper et al., (2000, 2007) have demonstrated that ATR alters the neuroendocrine control of the hypothalamic-pituitary-ovarian axis, a mode of action that is reflected by a disruption of the pre-ovulatory surge of luteinizing hormone (LH) in female rats (Cooper et al., 2000; Cooper et al., 2007). More recent studies have shown that ATR can also alter the activity of the hypothalamic-pituitary-adrenal (HPA) axis as indicated by changes in circulating serum steroid concentrations. Dose-dependent increases in corticosterone (CORT) and progesterone (P4) release in both male (Laws et al., 2009) and female (Fraites et al., 2009) rats have been observed following a single exposure to ATR. Increases in serum estrone (E1) and estradiol (E2) in male (Stoker et al., 2000; Stoker et al., 2002; Modic, 2004) and E1 in ovariectomized female (Cooper, 2010) rats following ATR administration have been previously reported as well. Thus, due to the extensive number of